Loma Linda Radiobiology Program Leads NASA Program Project Investigating Central Nervous System Responses to Charged-Particle Radiation

Astronauts in space face several health risks as a consequence of long-term exposure to ionizing radiation. It has long been recognized that cancer and cataracts are among these risks, but recently NASA has identified an urgent need to establish the level of risk to the central nervous system (CNS) as well.

The CNS controls the body's homeostasis mechanisms via electrical and endocrine signaling networks. It is important, therefore, to all the organ and tissue systems it coordinates. Little is known, however, about its susceptibility to low and moderate doses of ionizing radiation in general, and to charged-particle radiation (cosmic rays and solar flares) in particular. One concern, similar to that for cancer and cataracts, is whether such long-term exposure will lead to accelerated CNS dysfunction syndromes such as Alzheimer's disease or stroke. Scientists also have another concern: will long-duration space missions produce in-flight performance deficits; that is, CNS changes that could impair astronauts' functional abilities and compromise their safe return? To address these issues, Loma Linda University scientists and their colleagues are beginning an experimental campaign that will use mouse models to study long-term effects of radiation on the CNS to provide a foundation for understanding functional and structural changes that may arise following radiation exposure over a relatively long time scale; that is, the life spans of the animal subjects.

New Program Project Research Grant

Loma Linda researchers John Archambeau, Xiao-Wen Mao, André Obenaus, and Michael Pecaut with colleagues from six other institutions have formed a team led by principal investigator Gregory Nelson. . They were awarded funding for a five-year NASA Specialized Center of Research (NSCOR) program grant entitled "Progressive Alterations of Central Nervous System Structure and Function Are Caused by Charged Particle Radiation." The other institutions represented include: Stanford Research International, Inc. (Polly Chang), The Scripps Research Institute (Thomas Krucker), University of California at San Francisco (John Fike), Universty of California at Los Angeles (Igor Spigelman), University of North Carolina at Chapel Hill (Weili Lin), and Washington University in St. Louis (Sheng-Kwei Song). The new collaboration begins in January 2004; the work involves irradiation protocols using the Loma Linda University proton synchrotron for protons, the most common component of cosmic radiation, and the NASA Space Radiation Laboratory (NSRL) at the Brookhaven National Laboratory for high-energy iron ions, the most important heavy ions found in space.

Research Strategy

The NSCOR collaboration has three main goals. The first step is to quantitate radiation-induced loss of component cells in the hippocampus, a CNS region associated with learning and memory. Prior work by others has focussed on the formation of necrotic brain lesions following very large doses of radiation, but has not quantified changes in cell populations with time. However, Loma Linda investigators, led by Dr. Archambeau, recently introduced a technique that quantifies cell populations by using stereology, an unbiased mathematical sampling method that avoids the subjective errors inherent in traditional histopathology. They applied their method to the

microvessels of the rat retina. Their stereological analysis of microvasculature in the rat retina established, for the first time, the time- and dose-dependent loss of endothelial cells *in situ* in a CNS tissue (the retina is an outpocketing of the mid-brain). The stereological methods will now be extended to the various subsets of cells in the mouse hippocampus (neurons, glia, endothelia and stem cells) guided by the time-dose relations obtained for the rat retina; this work will be conducted by investigators Archambeau, Fike, Mao, and Pecaut.

To complement the stereological measurements, investigators will also employ a novel technique whereby a polymer (plastic) is infused into the CNS vasculature. Subsequently removing the CNS parenchyma leaves a three-dimensional cast of the microvasculature that then will be mapped by x-ray tomography to determine topological changes, the order of connectivity and microscopic infarcts associated with amyloid protein deposits [Krucker]. These experiments will accomplish the investigators' first goal: quantifying the time- and dose-dependent changes in cellular composition and architecture that determine functional CNS capacity.

The investigators' second goal is to quantify the function(s) of CNS tissues. Investigators will control the type, magnitude, and time course of various stimulatory inputs to test CNS tissue plasticity; that is, the ability of CNS tissue to cope with changing environmental influences and to maintain appropriate output. The investigators' primary measurement of function will be extracellular electrical recordings that sample the electrical outputs of ensembles of nerves. Investigators will conduct the measurements on "brain slices," thick sections of half-brains (hemibrains) isolated rapidly from euthanized animals and kept in artificial cerebrospinal fluid. Electrodes will send time- and intensity-modulated streams of impulses to these cell ensembles and will record their outputs. The resulting relationships may then be correlated with rudimentary memory formation; the measurements will reflect proper underlying CNS tissue functions such as connectivity, action potential generation and conduction, and neurotransmitter formation, secretion, and uptake. Assessments of individual nerve membrane properties by "patch clamp" recordings will complement the measurements on multi-cell networks [Obenaus, Krucker, Spigelman].

To complement the cell and tissue-level techniques, the investigators also will use intact animals; with these, they will employ two non-invasive methods to evaluate brain function and the evolution of changes with time. Electroencephalograms (EEGs) will map macroscopic spontaneous electrical activity [Obenaus]. MRI also will be used; two state-of-the-art magnetization sequences will visualize and quantify local oxygen utilization (a marker of metabolic activity) and the movement of water along CNS white matter fiber tracts (structural integrity). These methods are referred to as "oxygen extraction fraction (OEF)" and "diffusion tensor imaging (DTI)," respectively [Lin, Song].

After the time-dose relations are established for normal brains, the NSCOR team will challenge the nervous systems, in living animals, to quantify the brains' overall performance under stress. Astronauts' ability to perform under stress, such as illness or injury, is an important potential complicating issue for space flight. In the experimental animals, the investigators will introduce a systemic shock mediated by the immune system in the form of a reaction to lipopolysaccharide (LPS); this challenge will be a surrogate for a bacterial infection [Pecaut]. A second strategy will employ a surrogate for Alzheimer's disease. Alzheimer's-like features can be produced in

rodents by injecting β-amyloid protein or by using a genetic variant of mouse that spontaneously develops the pathological changes associated with Alzheimer's. Measurements of irradiated rodents will determine whether radiation exposure affects the latency and severity of the disease-associated pathological changes in the normal and variant animals [Krucker].

The third goal of the program is to quantify molecular changes (biomarkers) that correlate with, or underly, the cellular and system changes. The biomarkers reflect the known biochemical actions of radiation (key events) and the stressors used to challenge the CNS. The most fundamental site of ionizing radiation damage is chromatin and its constituent DNA; unrepaired or misrepaired DNA damage restricts or reprograms cell functions. Therefore, the investigators will quantify the frequency and determine the structural spectrum of mutations in the *E. coli* β-galactosidase gene present in a transgenic mouse strain's CNS tissue at the time and dose values selected for earlier studies. Investigators also will characterize the radiation-modulated state of the tissue by using transcription profiling hybridization of a targeted set of 96 genes involved in cytokine signaling during inflammation [Chang, Pecaut]. Finally, the team will use magnetic resonance spectroscopy to detect and profile the time course of alterations in selected metabolites that reflect cell death, neurotransmitter synthesis, and membrane breakdown [Obenaus]. This process will provide a framework for later extrapolation to humans.

Summary

The CNS NSCOR program project represents the first comprehensive investigation of the response of a mammalian brain structure to charged-particle radiation. The data set will establish a baseline for more detailed future investigations and will provide an initial scientific basis for NASA mission designers to estimate the health risks to astronauts embarked on long-duration space flights.